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Expanding Our Understanding of Polyglutamine Diseases through Mouse Models

Minireview

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The polyglutamine neurodegenerative diseases—a group that so far includes spinobulbar muscular atrophy (SBMA), Huntington's disease (HD), dentatorubropallidoluysian atrophy (DRPLA) and the spinocerebellar ataxias (SCA1, 2, 3, 6, and 7)—result from the expansion of an unstable CAG trinucleotide repeat coding for polyglutamine tracts in the respective proteins. The CAG repeat tracts are polymorphic and vary in their instability: the *SCA7* gene, for example, may expand by hundreds of repeats in one intergenerational transmission, whereas the *SCA6* gene is relatively stable and the difference in length between the wild-type and mutant CAG tract is only three repeats. But in all these diseases, triplet repeats exceeding their normal range cause progressive neuronal dysfunction and death within 10–20 years after the onset of symptoms, with longer repeat tracts causing earlier age of onset and more severe disease. Curiously, only a specific subset of neurons is vulnerable in each of these diseases, despite the ubiquitous expression of the relevant disease proteins throughout the brain and other tissues. The normal functions of these proteins remain a mystery, except for the androgen receptor (SBMA) and the α_{1A} voltage-dependent calcium channel (SCA6). The importance of mouse models for studying polyglutamine pathogenesis will become apparent after a brief look at the issues that have become clearer, and in some cases more complex, through their use.

The First Mouse Model for a Polyglutamine Disease

Cerebellar atrophy with severe Purkinje cell degeneration is responsible for the SCA1 ataxic phenotype (Zoghbi and Ballabio, 1995). Wanting to study the Purkinje cell pathology at the root of this disease, Burright et al. (1995) expressed full-length human *SCA1* cDNAs with different numbers of CAG repeats in Purkinje cells using a Purkinje cell–specific promoter from the *Pcp2/L7* gene. The resulting mice highly express either a wild-type *SCA1* allele with 30 repeats (30Q) or an expanded allele with 82 repeats (82Q). The 30Q mice are indistinguishable from wild-type littermates, but adult 82Q mice (known as the B05 line) develop severe ataxia and progressive Purkinje cell pathology (Clark et al., 1997).

The first histologic abnormalities, detectable at P25, are cytoplasmic vacuoles. Loss of proximal dendritic arborization and dendritic spines becomes apparent at 5 weeks when the mice begin to show mild difficulty on the rotating rod; by the time 82Q mice are overtly ataxic (12–15 weeks), the dendritic arborization is mostly lost, the molecular layer is atrophied, and some heterotopic Purkinje cells have moved to the molecular layer (likely in an attempt to preserve synapses). Immunolocalization studies show that mutant ataxin-1 localizes to ubiquitin-positive nuclear inclusions (NIs) in the Purkinje cells of 82Q transgenic mice, as it does in affected neurons in SCA1 patients (Skinner et al., 1997). These NIs appear in 82Q mice as early as 3.5 weeks and are detectable in 90% of the Purkinje cells by 12 weeks. Cell death becomes significant only after 6 months of age, long after the phenotype has appeared. The axons of the murine Purkinje cells are not dilated like the human SCA1-affected cells, but this is the only pathologic difference between the two. The B05 mice thus provide a reliable animal model for SCA1—but leave unanswered the question whether it is the full-length mutant protein or merely the expanded glutamine repeat that induces polyglutamine diseases.

Polyglutamine Tract or Full-Length Protein?

The first attempts to answer this question were to come from SCA3 transgenic mice. SCA3, also known as Machado-Joseph Disease (MJD), is similar to SCA1 except that dentate neurons, rather than Purkinje cells, are the primary sites of cerebellar pathology. Basal ganglia involvement is also prominent. Ikeda and colleagues (1996) used *Pcp2/L7* to generate transgenic mice expressing either full-length or truncated versions of ataxin-3. Mice expressing full-length ataxin-3 with 79 glutamines, designated MJD79, developed no ataxia or pathological changes; neither did Q35C mice, which expressed a truncated form of ataxin-3 with a CAG tract of 35 repeats. In contrast, both Q79C mice (carrying truncated ataxin-3 with 42 amino acids C terminal to a CAG tract of 79 repeats) and Q79 mice (bearing a peptide containing 79 glutamines) became ataxic by 4 weeks of age. By 8 weeks, Q79C mice showed massive degeneration of all three layers of the cerebellum, which was reduced to about one-eighth of its normal volume.

It is noteworthy that the expanded polyglutamine tract is sufficient to cause neuronal death, and that its toxicity causes massive cell loss as opposed to the more progressive dysfunction seen, for example, with ataxin-1. Ikeda and colleagues (1996) proposed that cell-specific proteolytic cleavage of the mutant protein frees the toxic polyglutamine tract, which then induces cell death. There is no evidence yet, however, that ataxin-3 cleavage occurs in affected brain regions of MJD/SCA3 patients. Moreover, it is impossible to conclude that full-length ataxin-3 is not toxic without data on transgene expression levels from this series of mice.

During the same period, Mangiarini and colleagues (1996) generated mice using a 1.9 kb human genomic fragment containing huntingtin (*HD*) 5′ flanking sequences and exon 1 with an unstable expanded CAG tract of ~130 repeats. The R6/2 line (with 144 repeats) ubiquitously expresses the first 69 amino acids of huntingtin with the elongated CAG tract at lower-than-endogenous levels. Expecting to study CAG repeat instability in the mouse, the investigators were somewhat surprised to discover that this small fragment was enough to create a progressive neurological phenotype

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comprising some HD-like features. R6/2 mice are indistinguishable from nontransgenic littermates at weaning, but at 9–11 weeks show handling-induced seizures, limb dyskinesia that progresses to reflexive clasping of the limbs to the body when suspended, tremor, and stereotypic involuntary movements. As the disease worsens, the mice unaccountably lose body weight, develop urinary incontinence and unusual vocalizations, and die suddenly of unknown causes between 10 and 13 weeks. Despite the neurologic signs, neuropathology revealed only that R6/2 brains are consistently smaller than normal and develop NIs throughout the CNS, prior to any neurobehavioral changes. These amembranous NIs show a filamentous morphology by electron microscopy (EM) and stain positively for ubiquitin (Davies et al., 1997). None of the neurodegeneration typical of HD is apparent; it may be that the mice do not live long enough to develop HD pathology. It is somewhat surprising that R6/2 mice show some features typical of HD, since the transgene codes for only 3% of huntingtin. Does the truncated peptide retain the full-length protein's ability to interact with certain cell-specific factors, or does the expanded polyglutamine tract produce a generalized toxicity that overlaps with some features of HD?

To see whether any gene with an expanded CAG repeat might cause pathology, Ordway and colleagues (1997) introduced a 146-unit repeat into the mouse hypoxanthine phosphoribosyltransferase (*Hprt*) gene. These knockin mice, like the R6/2, acquire seizures, tremors, and die prematurely; they also develop NIs, without neurodegeneration. In contrast to the R6/2 line, however, they gain weight. The expanded polyglutamine tract is clearly toxic independent of protein context, but it seems that context mediates the phenotypic differences and selective neuronal vulnerability of the polyglutamine diseases.

Further support for this hypothesis comes from mice generated by Reddy and colleagues (1998), who expressed full-length *HD* cDNA with 16, 48, or 89 CAG repeats at high (two to five times endogenous) levels under the control of a cytomegalovirus promoter. Mice with 48 or 89 repeats develop progressive neurobehavioral abnormalities. At 8 weeks, they begin clasping; at 20 weeks, they are hyperactive; by 24 weeks, the mice develop urinary incontinence and decreased locomotor activity, which progresses to akinesia in 4 to 5 weeks. Once volitional movements cease, the mice do not respond to sensory stimuli and die within a week. Striatal degeneration, including human HD-like apoptosis and gliosis, becomes evident during the hypokinetic phase. Apoptosis also occurs in other areas typically affected in HD, supporting the notion that selective neuronal vulnerability requires the context of full-length huntingtin. Furthermore, transgenic mice expressing full-length HD develop most of the phenotypic features at a much later time (20 weeks) than R6/2 mice (9 weeks), even though the former have much higher levels of huntingtin expression.

In sum, the expanded polyglutamine tracts by themselves wreak havoc, perhaps by misfolding or otherwise disrupting nuclear functions. The size of these fragments, usually $<$50 kDa, likely facilitates their transport into the nucleus; this would explain the high toxicity of truncated ataxin-3, the 3% fragment of huntingtin, and the HPRT protein (only 48 kDa with the polyglutamine). The full protein context may mitigate the toxicity of an expanded tract, slowing symptom onset; it may also lead to gains of function through new protein–protein interactions, thereby mediating selective toxicity.

Nuclear Inclusions Revisited

The finding that, in transgenic mice, huntingtin localizes to nuclear inclusions (Vonsattel and DiFiglia, 1998) rekindled interest in similar observations made 20 years previously in human HD patients. Is the fact that NIs appear not only in HD but in SBMA, SCA1, SCA3, SCA7, and DRPLA patients significant (Paulson, 1999)? It seems intuitive that insoluble aggregates might disrupt nuclear functions. But it is equally conceivable that NIs might benefit the cell by sequestering the mutant proteins. Consistent with this latter possibility is the finding that, in the mouse model expressing full-length HD, NIs appear in several brain regions typically spared in HD (e.g., Purkinje cells). In fact, the frequency of inclusions is $<$1% in the striatum, where apoptosis is most conspicuous (Reddy et al., 1998).

Is the nuclear location of the protein significant? Working with SCA1 models, Klement et al. (1998) set out to determine if ataxin-1 must be in the nucleus to cause disease. They generated transgenic mice that express expanded ataxin-1 (82 glutamines) with a mutated nuclear localization signal (NLS), causing ataxin-1 to remain diffusely distributed throughout the cytoplasm. Although these mice express ataxin-1 in Purkinje cells at levels comparable to those observed in the original SCA1 (82Q) transgenic mice, they develop neither Purkinje cell pathology, motor dysfunction, nor aggregates. Nuclear localization of ataxin-1 is thus critical for pathogenesis and for aggregation.

But are pathogenesis and aggregation necessarily linked? Klement et al. (1998) characterized transgenic mice that express mutant ataxin-1 (77Q) lacking the self-association region, found to be essential for ataxin-1 dimerization. These mice develop ataxia and Purkinje cell pathology similar to 82Q mice, but without apparent nuclear ataxin-1 aggregation. Deletion of 122 amino acids could compromise the protein in various ways (folding, turnover rate, interactions), but this truncated ataxin-1 retains its ability to interact with its known partner, LANP, and produces all the neurobehavioral and unique pathologic features observed in 82Q mice. It wields the same pathogenicity as full-length expanded ataxin-1, despite its inability to aggregate. Therefore, although nuclear localization of ataxin-1 is necessary, nuclear aggregation of ataxin-1 is not required to initiate pathogenesis in transgenic mice.

It remains unclear whether NIs are mere epiphenomena, beneficial to the cell, or participants in disease progression. The findings of Cummings et al. (1998) that NIs stain positively for the proteasome and molecular chaperones suggests that they contain misfolded proteins targeted for proteolysis; enhancing molecular chaperone or proteasome activities may facilitate processing of the mutant proteins and provide an opportunity for therapeutic intervention.

Polyglutamine Toxicity in the Context of Proper Spatial and Temporal Expression

It was now established that overexpression of mutant proteins induced neurodegeneration in mice. The next

logical step was to determine whether expressing near-endogenous levels of the proteins would prove pathogenic.

Hodgson and colleagues (1999) generated HD transgenic mice that replicate the disease-causing mutation in the same developmental and tissue-specific pattern seen in human HD patients. They used yeast artificial chromosomes (YACs) with the complete human *HD* locus carrying 18, 46, and 72 CAG repeats (corresponding to normal, adult-onset-, and juvenile-onset-causing tracts in humans) to create the so-called YAC18, YAC46, and YAC72 lines. No developmental, neurobehavioral, or pathological abnormalities are apparent in YAC18 and YAC46 mice, but the YAC72 mice become hyperactive. Both YAC46 and YAC72 mice develop progressive electrophysiological dysfunction. Interestingly, the YAC72 mice show these changes prior to nuclear localization of huntingtin. Immunostaining with an N-terminal antibody reveals increased huntingtin expression in striatal neuron nuclei; hyperchromasia and striatal atrophy, but no NIs, are visible by EM.

At 6 months, YAC72 mice show NMDA receptor hyperactivity, providing in vivo evidence of excitotoxicity in early HD pathogenesis. At 10 months, neither YAC46 nor YAC72 mice showed long-term potentiation (LTP) after-high frequency stimulation at Schaeffer collaterals. To explain this puzzling result, the authors note that increased NMDA receptor function would lead to elevated calcium influx during synaptic transmission and that increases in intracellular calcium levels correlate with calcium-dependent desensitization of NMDA receptors. Hodgson et al. (1999) speculate, then, that during high-frequency stimulation, the NMDA receptors may be inactivated by the high calcium influx, leading to loss of LTP in the mutant mice. The electrophysiological abnormalities precede any neurobehavioral or neuropathologic changes. Although the time at which these abnormalities first appear needs to be determined, the data suggest that cytoplasmic functions (e.g., neurotransmitter behavior and calcium regulation) are affected early in HD pathogenesis. Translocation of huntingtin to the nucleus is evident only in the medium spiny neurons, which are susceptible to neurodegeneration; this may account for the selective neuronal vulnerability seen in HD.

The most accurate mouse models for HD or other polyglutamine diseases should be those carrying CAG expansions within the endogenous genetic locus, i.e., knockins. Two such mouse mutants carrying expanded CAG repeats of different lengths in the mouse *Hd* locus were generated by gene targeting. One line carrying 50 CAG repeats has developed no neurological, pathological, or physiological changes, although the mutant protein is expressed at wild-type levels and rescues the embryonic lethality of the *Hd* null mutation (White et al., 1997). In a second allele of *HD* knockin mice, an expansion of 72–80 CAG repeats was introduced into the *Hd* locus (Shelbourne et al., 1999; Usdin et al., 1999). Extensive analysis revealed few abnormalities besides aggressive behaviors in 3-month-old mutant mice and impaired LTP at Schaeffer collateral–CA1 synapses. Reduced posttetanic potentiation and paired-pulse facilitation suggest that the mice may be unable to sustain neurotransmission during repetitive stimulation. Although some of the phenotypic changes do not directly correlate with an HD phenotype, alterations in synaptic plasticity and subtle emotional disturbances may precede the motor and cognitive abnormalities typical of HD.

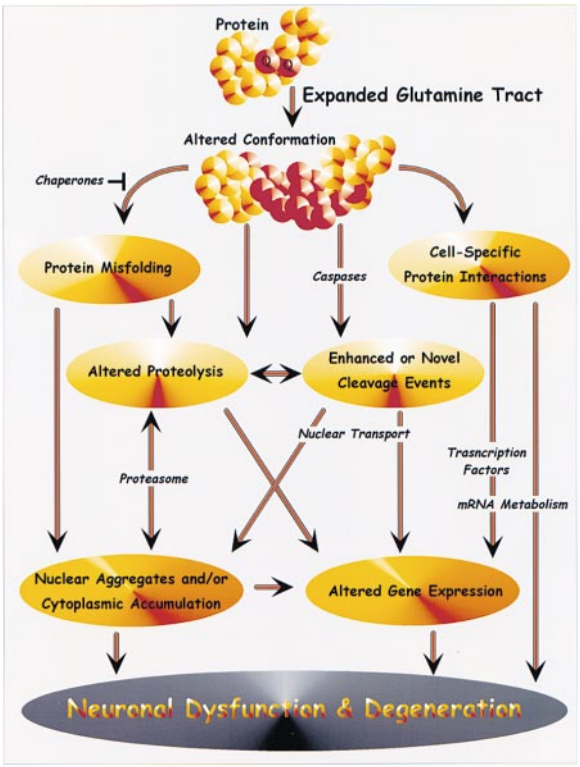

Figure 1. Model for the Pathogenesis of Polyglutamine Diseases

A review of the various mouse models makes it clear that appropriate spatial and temporal expression of a full-length protein with an expanded glutamine tract is insufficient to produce overt phenotypes. Mice that manifest symptoms paralleling human disease (SCA1/B05, HD full-length transgenics) typically overexpress the mutant protein. From this, we infer that pathogenesis is, at least in part, a function of two factors: mutant protein levels and the amount of time a neuron is exposed to the mutant protein (a matter of decades in humans).

Remaining Questions and Future Directions

The above mouse models strongly support the notion that polyglutamine pathogenesis is caused by some toxic function gained by the mutant protein as a result of the expanded CAG repeat. Selective neuronal vulnerability may be determined by the protein context of the expanded polyglutamine tract and the level of protein in those cells. Mutant proteins may be abnormally processed or interact abnormally with other proteins, which presumably contribute to selective neuronal dysfunction and degeneration (Figure 1).

Is the pathogenesis of all polyglutamine disorders mediated by the same mechanism(s)? The answer is most likely "yes and no." Protein misfolding and turnover seem to be shared features, since glutamine tract

expansion leads to the accumulation of mutant protein in all the diseases evaluated. The variation in sites of accumulation of the mutant protein, however—in SCA2 and SCA6, the mutant proteins accumulate in the cytoplasm, not the nucleus (Huynh et al., 1999; Ishikawa et al., 1999)—implies that the downstream pathogenic mechanisms may differ. Polyglutamine expansion could cause the mutant proteins to adopt altered conformations, leading to their ubiquitination, aggregation, and resistance to proteasomal degradation. Over the course of the disease, the ubiquitin–proteasome system could become encumbered by the aggregate-prone proteins, which in turn could alter the turnover of other critically short-lived proteins. Although neuronal dysfunction and cytoplasmic changes could occur very early in the pathogenesis, later-stage disruptions of nuclear structures and/or functions might be common factors in several polyglutamine diseases.

Even a unifying model for polyglutamine-induced neurodegeneration, however, leaves a number of questions unanswered including the following key issues. What cell-specific determinants underlie the selectivity of neuronal degeneration: cell-specific interactors or cell-specific alterations of gene expression? Are the functions gained by the mutant proteins novel or intrinsic? What are the earliest physiological changes in affected neurons? Is there therapeutic potential in inhibiting cleavage or nuclear transport of the mutant proteins?

Present and future generation animal models—mouse, *Drosophila*, and *C. elegans* alike—will be invaluable tools in addressing many of these questions. For example, the significance of interacting proteins in selective neuronal vulnerability can now be studied by mating the various models with animals that either lack or overexpress the interactors in the vulnerable neurons. The emerging principle that truncation of the parent protein is an important step in the pathogenesis of several of these diseases can now be tested by investigating the posttranslational processing of these proteins in vivo. Ona et al. (1999), for example, recently showed that caspase-1 is activated in the R6/2 mice, and inhibiting its activity reduces endogenous huntingtin cleavage. Furthermore, this causes several of the phenotypes described above to progress more slowly. It would be interesting to see if inhibition of caspase-1 would also modify the phenotype of mice expressing full-length huntingtin. Lessons learned from in vitro studies—e.g., ataxin-1 aggregation in transfected HeLa cells can be suppressed by overexpressing a molecular chaperone—can now be carried into mouse models to determine whether these modifications occur in vivo. Likewise, the putative involvement of the ubiquitin–proteasome pathway might be investigated by mating the polyglutamine mouse models with mice that have deficiencies in this pathway or, conversely, with transgenic mice overexpressing factors within the pathway. Most importantly, mouse models allow the study of early phenotypic changes for which patient material is seldom available. Gene chips and other array technologies should prove invaluable for finding transcriptional changes early in the disease process. Characterizing changes that occur prior to any pathological events will greatly expand our knowledge of the molecular mechanisms that cause neurodegeneration in polyglutamine diseases.

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